THE EFFECT OF THIOUREA, ADMINISTERED BY IMMERSION OF THE MATERNAL ORGANISM, ON THE EMBRYOS OF LEBISTES RETICULATUS, WITH NOTES ON THE ADULT GONADAL CHANGES [1]

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The terms oviparous, viviparous and ovoviviparous are commonly found in the literature discussing embryology and physiology. Oviparous and viviparous may be defined with relative ease and the interpretations of most authorities are in agreement. Briefly, oviparous refers to animals that deposit eggs outside of the body, while viviparous applies to animals that retain the ova and have their development within the soma. The latter term is applied to those forms having highly specialized structures, such as placentae, which attach the developing young to some part of the reproductive system of the adult in order to provide an avenue of material exchange, including nutritive materials for the growth of the embryo during the greater part of its development within the parent. The third term, ovoviviparous, has been variously defined and is less definite. It is agreed that in those forms described as being ovoviviparous the young are retained within the maternal reproductive system and leave the mother's body fully formed, viable and without any surrounding membranes. There does not appear to have been a permanent attachment, however, and the degree of dependence of the young on the parent is a moot question. The eggs of many species referred to as ovoviviparous are macrolecithal, and many authors believe the produced egg contains enough yolk to meet the nutritive needs of the developing young until it leaves the parent. The young is materially independent of the adult and uses the latter only as a harbor while developing. The distinction between viviparity and ovoviviparity is therefore classically based on anatomical and physiological differences.

A true anatomical uterus and placenta, which permits a firm attachment of the embryo to the parent, is wanting in teleosts. Structures and modifications of parts of the reproductive tracts (of both male and female) which permit retention and internal hatching of the young have been reported to occur in diverse orders of the class (Turner, 1942, 1947). In some of the species studied it has been shown that both parent and young may possess structures whose histological and cytological make-up, spatial arrangement and time of appearance suggest a material exchange between parent and young (Blake, 1867, 1868; Eigenmann, 1892; Fraser and Renton, 1940; Lane, 1903; Mendoza, 1937, 1938, 1939, 1940, 1941, 1943; Purser, 1938; Turner, 1933, 1936, 1937a, 1938a, 1938b, 1940a, 1940b, 1940c,

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1940d; Wyman, 1854). Some physiological studies (Bailey, 1933; Scrimshaw, 1944, 1945) comparing egg and embryo weights indicate the supply of materials to the young by the parent in certain species of teleost.

This paper reports the results of a study designed to reinvestigate the question of exchange between parent and young in one teleost, the guppy, *Lebistes reticulatus* (Peters), often referred to as ovoviviparous, by using the anti-thyroid chemical, thiourea, which is known to have the ability to cause the alteration of the morphology and physiology of the thyroid gland. It was thought that the chemical, when supplied to the adult, would produce discernible changes in the young if a material transfer does exist. *Lebistes reticulatus* was used as the experimental animal because of its hardiness, macrolecithal egg, and the presence of structures (Purser, 1938) that could possibly aid in the transfer of substances.

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Materials and Methods

Seventy-five brood-bearing *Lebistes reticulatus* were utilized in this work. They were selected from a larger group of females exposed continuously to males soon after birth up to and one day after the delivery of their third brood. The fish were all of approximately equal size and found to present young at 28–31-day intervals. They were divided into 5 groups of 15 each as follows:

Group C: Received no treatment, served as controls.

Group 1D: Placed in a 0.04% thiourea solution one day after the delivery of their third brood. This timing exposed the adults to the solution at the time a group of ova were maturing, during fertilization and the entire ontogeny of the young.

Group 1W: Placed in a 0.04% thiourea solution one week after the delivery of their third brood. This timing exposed the adults to the solution at approximately the time of fertilization and during the entire ontogeny of the young.

Group 2W: Placed in a 0.04% thiourea solution two weeks after the delivery of their third brood. This timing exposed the adults to the solution during the latter two-thirds of the ontogeny of the young.

Group 3W: Placed in a 0.04% thiourea solution three weeks after the delivery of their third brood. This timing exposed the adults to the solution during the last one-third of the ontogeny of the young.

The young of the groups were labeled with a "Y" after the parents' group, giving the symbols CY, 1DY, 1WY, 2WY, 3WY.

Homotypic conditioned water was used in the preparation of the thiourea solutions and in the tanks containing control fish. Each animal was kept separate in a one-gallon container without plants, "scavengers" or aeration. Solutions were changed semi-weekly. All fish, except as noted below, were allowed to present their broods. To insure against a long exposure of the newborn young to the thiourea solution all tanks were checked four times a day and any young found were fixed immediately. In only two cases, were broods presented during the night. These young may have been in the solution for a maximum of 10 hours. Seventeen non-control fish were observed to be delivering young and were sacrificed during the birth process. All other adults were killed when young were found in the tanks. Water temperatures ranged from 72 to 78° F. throughout the experiment. A portion of the controls and of each of the treated groups coexisted at all times. This arrangement served as a check on possible seasonal effects. Tanks containing control fish were always adjacent to the tanks containing treated fish in order to assure an equal distribution of light to all. Daylight was supplemented with artificial light during the late fall and winter months.

The fish were killed and fixed in Bouin's solution. As soon as movement ceased the adults were removed from the liquid, their abdominal wall was slit and they were reimmersed in the agent. This procedure allowed a more rapid and direct penetration of the fixing fluid into the body cavity. Adult fish were decalcified with nitric acid plus phloroglucin; young were not decalcified. Xylol was used as the clearing agent in preparation for embedding in 56–58° C. Histowax. Sagittal serial sections of the entire fish were cut at five micra and stained either with Harris' hematoxylin and eosin or a modified Masson procedure.

Thyroid cell height was measured with a calibrated ocular micrometer. A minimum of 100 cells was observed for each fish, involving as many different follicles as possible. The data obtained were subjected to statistical analysis and their significance determined.

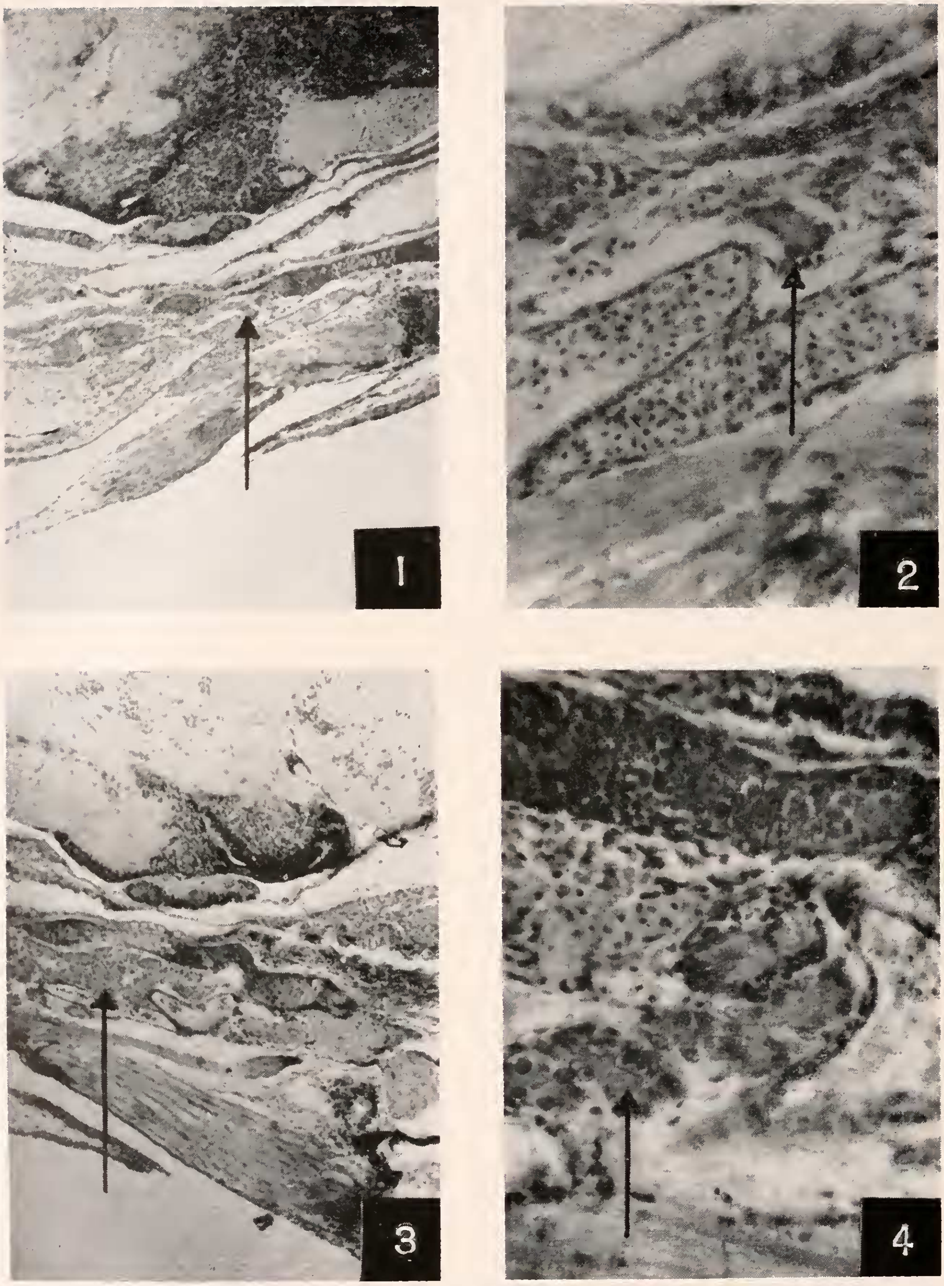
Brood sizes and intervals of all individuals of all groups were recorded. The ovaries of all adult fish were examined macroscopically and microscopically and the contained ova were grouped according to size.

Results

I. *Thyroid*

A. *Normal.* The thyroid gland of the normal *Lebistes reticulatus* was found to be a diffuse and unencapsulated organ consisting of many separate follicles in the connective tissue of the floor of the mouth. They are arranged alongside the ventral aorta and other blood vessels and extend from the anterior part of the mandible to the gill region; see Figures 1 and 2. Thyroid epithelia were not found in any other part of the body. Without exception the follicular cells are of a squamous or a very low cuboidal nature, as seen in Figure 5. The contained colloid is dense, homogeneous and fills the interior of all the follicles. The picture is essentially the same for the adult and the newborn.

B. *Treated Adults.* After immersion in the thiourea solution, hyperplastic changes were noted in all fish. Follicles increased greatly in size with very great variation. Without exception, follicles increased in number and extent. Some were found posteriorly to the level of the heart, throughout the connective tissue of the entire lower jaw, crowded around the blood vessels, and in the gill region. Members of group 1D showed the greatest degree of metastasis. In this group, thyroid cells were found in the myocardium in 12 cases, in the kidneys in 9 cases, in



Tissues represented by Figures 1–7 were stained with Harris' hematoxylin and eosin. Figure 8 represents an unfixed, unstained young.

Plate I

FIGURES 1 and 2. Section of the floor of the mouth of a normal young showing few thyroid follicles. Figure 1, × 132; Figure 2, × 525.

FIGURES 3 and 4. Section of the floor of the mouth of a young from a thiourea-treated fish showing massing of cells and afollicular arrangement of cells near the blood vessels. Figure 3, × 132; Figure 4, × 525.

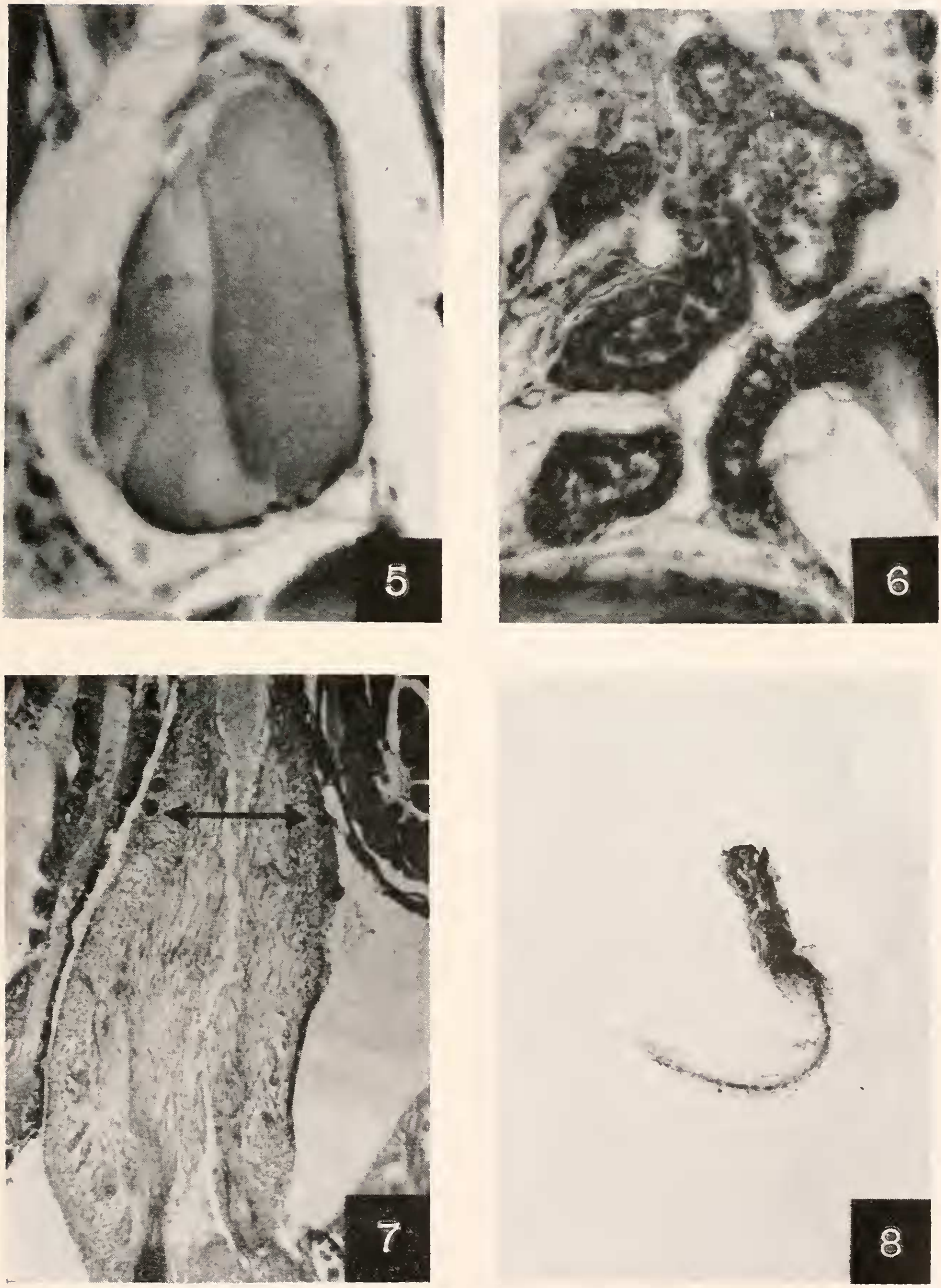


Plate II

FIGURE 5. Thyroid follicle from an untreated fish. × 525.
FIGURE 6. Thyroid follicle from a thiourea-treated fish. × 525.
FIGURE 7. Heart of a thiourea-treated fish showing follicles in the myocardium. × 132.
FIGURE 8. Aborted young from a thiourea-treated fish. × 15.

the intestines and spleen in 8 cases. Scattering of thyroid tissue took place to a lesser extent in fish of groups 1W and 2W. Group 3W did not show any atypical location of thyroid cells. The invading tissue in the new areas was arranged in small follicles or was massed in layers approaching an afollicular disposition. Colloid was reduced in amount. It was of an acidophilic nature and appeared foamy and vacuolated.

Thyroid cell height was markedly increased, the amount increasing with the time of exposure (Fig. 6). In all cases, the difference between the experimental and control animal data proved to be statistically significant (see Table I). Cytoplasmic staining was more intense. The nuclei increased in size, became more vesicular and basal in position. Nucleoli became more prominent.

Table I

Experimental condition of the fish and thyroid cell height

State of immersion	Mean thyroid cell heights (μ)	S.E.*
Control—adults	2.71	0.010
Young male	2.22	0.008
Young female	2.33	0.009
1 day—adults	7.21	0.031
Young male	9.66	0.021
Young female	9.81	0.027
1 week—adults	6.70	0.032
Young male	9.55	0.022
Young female	9.69	0.021
2 weeks—adults	6.52	0.019
Young male	9.04	0.017
Young female	9.22	0.019
3 weeks—adults	6.17	0.014
Young male	8.60	0.020
Young female	8.84	0.015

* Standard error (standard deviation of the mean): $S.E. = \sqrt{\frac{Ed^2}{n(n-1)}}$.

C. *Young from treated adults.* All the young from all groups showed extensive invasion of new areas by thyroid cells. The gill regions, heart, kidneys, spleen and intestines contained thyroid tissue. Myocardium invasion is seen in Figure 7. No one specimen showed metastasis to all sites, however. Follicular arrangement became the exception rather than the rule. Cells were massed in large groups without follicular arrangment, as seen in Figures 3 and 4. Colloid was scant or, in many cases, absent. Thyroid cell height was increased and in all cases proved to be significantly different from the controls.

II *Gonads*

A. *Normal adult.* It can be seen macroscopically as well as with a dissecting microscope that after the presentation of a brood the ovary contains a large number of ova. Study and measurements showed that they could be arranged in groups. The oldest and most mature group consists of eggs measuring 0.8 to 1.5 mm. These are orange in color and rather transparent. In numbers they approximate the size of the last brood or may be slightly more numerous. A second group consists of ova measuring 0.3 to 0.5 mm. These immature eggs, in addition to being smaller, are white, dense and opaque. Their number is considerably larger than either the first group or the brood last presented. Eggs in a still more immature stage of development are also present. They are by far the most numerous. Schematic grouping of the latter is not feasible because of too great a variation in size, almost continuous gradation existing within this group. Definite intervals existed between the three major groups, however.

B. *Normal young.* Sexual distinction of newborn *Lebistes* cannot be accomplished macroscopically. The anal fin modification (the gonopodium) and the typical coloration is not obtained by the male for nearly two months. Histological study of the immature gonad does reveal the sex of the individual, however, and may be used to sex specimens.

Table II

Experimental condition of the fish and brood size, brood interval and ova development

	Brood size		Brood interval in days		0.8–1.5 mm. ova		0.3–0.5 mm. ova	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Control	14.6	0.25	29.5	0.08	15.3	0.23	20.3	0.33
1 day	8.5	0.25	35.0	0.23	4.6	0.12	1.8	0.07
1 week	9.5	0.23	33.0	0.07	6.1	0.18	2.2	0.08
2 weeks	10.6	0.20	32.0	0.06	6.8	0.13	3.2	0.08
3 weeks	14.2	0.26	30.8	0.07	12.6	0.25	12.5	0.26

C. *Treated adult.* There is a very apparent decrease in gonadal activity. Without exception, all members of groups 1D, 1W and 2W presented a brood smaller in number than either of their previous broods or the average of their three previous broods. This is most evident in group 1D. Members of group 3W were not so affected, as shown in Table II. Three of the 15 members of group 1D and one member of the 15 in group 1W presented their young earlier than the expected date, that is, the date calculated from their previous performance and the average for the group. The aborted young were not viable. Their bodies were blackened and were undergoing resorption (see Figure 8). A statistically significant increase in the interval between the presentation of broods in a normal manner took place after exposure to thiourea (see Table II). The ovaries of members of groups 1D, 1W and 2W contained fewer than the anticipated number of large, orange, transparent ova. Their number was smaller than the number of young in the broods presented by the fish since the start of treatment with the anti-thyroid drug and smaller than either of its pre-thiourea broods. In addition, the number of ova of the 0.3-0.5 mm. group was markedly reduced. Ovaries of all controls contained more small ova than either the number of the young born in a brood or the eggs composing the more mature group. These data are included in Table II.

D. *Young from treated adults.* The gonads of all young from treated adults were developed sufficiently to permit sexing of the individual by means of histological examination.

Discussion

It has been known for a long time that some teleost fishes retain their young within their body. This live-bearing habit is found in widely distributed families. According to Turner (1947), there seems to be no relation to ecological conditions, geographic distribution or taxonomical relationship. Live-bearing species are found in cold or warm water, lagoons, swift running streams, lakes, caves, at muddy bottoms, at water surfaces and among rocks in fresh or salt waters throughout the world. Although the group of teleosts is old and has had time for evolutionary change, none of the species has evolved a true placental attachment, erosion of maternal tissue by the embryo or an anatomical uterus to harbor the young. The length of time of retention of the young is variable. Some species (for example, the scorpaenids) present their broods in a very immature state. Turner (1942) referred to this condition as "incipient viviparity." Other species present their young in a more developed state. Males of *Cymatogaster aggregatus* are sexually mature when born. The number of broods developing at any one time depends upon the species in question. Superfetation is found in a number of groups. In the extreme case of *Heterandria formosa*, as many as nine broods in different stages of development may be contained within the ovary. All of the live-bearers, of course, show internal fertilization. The sperm traverse the gonaduct to reach the ovary. Egg activation and fertilization may take place immediately or the sperm may be immobilized and stored for a period of months in the ovary and used to fertilize several succeeding broods, according to van Oordt (1928) and Purser (1938). In the latter case, part of the ovarian epithelium acts as a nurse cell. No rule can be stated to describe the site of fertilization, time of ovulation, or area utilized to contain the developing young. If fertilization is intrafollicular, the development may proceed either within the follicle, or the contents of the follicle may be evacuated into the ovarian cavity, or the zygote may be extruded immediately. Turner (1947) referred to the first type as "follicular gestation," the second as "ovarian cavity gestation." Both of these necessarily mean that ovulation follows the fusion of the egg and sperm. Superfetation is found to be superimposed upon follicular gestation; in this case all follicles are not emptied at the same time. Hatching may take place in the follicle, ovarian cavity or after expulsion from the adult's body.

There is no correlation between site of harboring and the amount of yolk contained in the egg. The deutoplasm may be abundant or relatively scarce in either follicular gestation or in ovarian cavity gestation. Neither has the amount of yolk been found to be correlated with gestation time. The yolk may be relatively abundant during a short gestation period or it may be at a minimum while the gestation period is lengthy. The latter finding, coupled with the existence of temporary structures (both maternal and embryonic) present only during the gravid condition, as well as changes in permanently established organs, suggests *a priori* a material exchange between adult and embryo may proceed.

Structural developments and organ changes of some kind and degree have been found to exist in all live-bearing fish so far studied, regardless of egg yolk contents.

In forms showing a macrolecithal condition and displaying follicular gestation, maternal changes are mainly a thinning of the follicle wall and greatly increased vascularity of that area. The embryonic vascular pericardial sac usually becomes enlarged and drawn over the head region to form a hood-like covering which definitely increases the embryonic vascular surface (Purser, 1938; Turner, 1940c). On the other hand, species whose embryos are equipped with a small yolk supply show a greater number and degree of modifications in both parent and young. The follicular lining, in addition to becoming highly vascular, develops a large number of vascular extensions very much like villi. These may make contact with and be closely applied to the surface of the embryo or be suspended in the fluid contained in the follicle. The pericardial sac is more highly expanded than in the previous case.

Species utilizing the ovarian cavity as the embryo-harboring site display a number of more complex structures. Following fertilization and while still within the follicle, the embryos possess for a short period of time the distended pericardial sac described for the follicular gestation embryos. When in the ovarian cavity the structure is lost and is replaced by extensions of the anal area called trophotaeniae (Mendoza, 1937; Turner 1933, 1937a). In the developing embiotocid fishes, the soft tissues between the ray ends of the vertical fins become vascular and extended (Blake, 1867, 1868). At this time the adult embiotocid cavity wall develops a number of vascular flattened processes which may become applied to the surface of the embryo gill or mouth area. The epithelial lining has been described as high columnar and secretory at this time (Mendoza, 1939, 1940). The spatial arrangement of these structures, the time of their appearance, and their structure certainly support the hypothesis of material exchange in the possessor. Turner (1940) has employed the term "pseudo-placentae" to describe them. The fluid within the cavity which bathes the embryos is said to contain degenerating cells and non-surviving embryos. Both the fluid and these cells may serve as a source of food materials (Turner, 1937a, 1947).

Scrimshaw attempted to provide more definite proof for an interchange of substances. He determined the dry and wet weights of embryos at various stages of development. In 1944, he found that the dry weight of the *Heterandria formosa* embryo increases from 0.017 mg. at the time of fertilization to 6.8 mg. at the time of birth. Wet weight measurements were not sufficiently accurate, for no way was found accurately to eliminate the excess water. Scrimshaw in 1945 applied this method of attack to many genera of Poeciliidae. He found that the dry weight of the intact follicle, determined at various stages of development, showed no significant weight change in 18 species. This is in marked contrast to the situation in oviparous fish where approximately one-third of the initial weight of the egg is used for maintenance during development. Accordingly, Scrimshaw concluded that in the species studied, the adult contributes positively to the embryo and that they receive as much nourishment quantitatively as they require for maintaining their metabolic rate.

In the present study, *Lebistes reticulatus*, a live-bearing Poeciliid was utilized. This species displays follicular gestation and the embryos are supplied with much yolk. *Lebistes* is often referred to as ovoviviparous and it is thought by some workers that the material exchange is nil or at most is restricted to gases. Purser (1938) studied *Lebistes* and found an extremely vascular area covering the yolk sac and extending between the eyes and opercula and making its way over the head. This strap of tissue is almost completely covered with a vascular network. Purser referred to this development as a "specialized respiratory mechanism." The present writer confirms the head covering and records ridges containing blood vessels on the internal surface of the follicular wall.

Anti-thyroid compounds such as thiourea and related substances have been shown to affect the thyroid in all classes of vertebrates. The usual finding is an increase in the size of the gland and its cells and the production of a hypofunctioning organ. Studies have included many animals, among which are the following: rat (Astwood *et al.*, 1943; Mackenzie and Mackenzie, 1943; Hughes, 1944), monkey (McGinty and Wilson, 1949; Mixner *et al.*, 1944; Astwood *et al.*, 1944), reptiles (Ratzersdorfer *et al.*, 1949; Adams and Craig, 1949, 1950, 1951), amphibians (Gordon *et al.*, 1943, 1945b; Hughes and Astwood, 1944; Joel *et al.*, 1949; Adams and Craig, 1949), fishes (Goldsmith *et al.*, 1944; Nigrelli *et al.*, 1946; Lever *et al.*, 1949; Rasquin, 1949; Osborn, 1951; Chambers, 1951; Scott, 1953).

Goldsmith *et al.* (1944), reported hyperplasia of the thyroid in a hybrid strain of *Xiphophorous helleri* and *X. maculatus* after immersion of the fish in a thiourea solution. Their fish were inhibited in growth and failed to develop secondary sex characters. The studies of Rasquin (1949) on *Astynax mexicanus* and Scott (1953) on *Brachydanio rerio* substantiate the hyperplasia previously reported. Lever *et al.* (1949) investigated the effect of a short thiourea treatment upon the thyroid of *Lebistes reticulatus* and *Callionymus lyra*. They reported an increase in thyroid cell height as early as the first week. Fish in the present study showed extreme hyperplasia within eight days, but checks at shorter intervals were not made. In addition to the localized changes, metastasis to many areas of the body was induced.

In many animals, a hypofunctional state of the thyroid may be produced surgically or chemically by a number of goitrogenic compounds. The relationship between oxygen consumption and the metabolic rate is understood and known to be affected by the thyroid status in homoiothermal animals. Hypofunction leads to decreased oxygen consumption while hyperfunction results in the increase of oxygen consumption. Attempts to alter the oxygen uptake in fish by methods which have proven to be effective and reliable in other animals have yielded results that are contradictory. Drexler and Issekutz (1934) found that thyroxine in a 1 : 500 000 concentration failed to increase the metabolic rate of *Lebistes*. The work of Etkin *et al.* (1940) failed to reveal any increased metabolism after the feeding of desiccated thyroid tablets to goldfish for 9 weeks; the body weights were not changed even after treatment for 16 weeks. They interpreted the results as an absence of a metabolic effect of the thyroid in fish. Grobstein and Bellamy (1939), working with immature *Xiphophorus* (=*Platypoecilus*), produced an exaggerated exophthalmos, a change in body proportions and precocious sexual development. Smith and Everett in 1943 administered thyroxine and desiccated thyroid to new-born guppies for 50 to 90 days. They found no change in growth rate and the fish differentiated at the same time as the controls. Further, oxygen utilization was not different after a one-week exposure.

The administration of anti-thyroid drugs to fish has produced more consistent data. Goldsmith *et al.* (1944) considered the inhibition of growth and retarded sexual development as (p. 133) "presumptive evidence that the thyroid gland is concerned with growth and development of sex characters of fishes." They concluded, "it appears that thiourea produces these effects by interfering with thyroid hormone production in much the same manner as reported for the mammal" (p. 133). Scott (1953) ascribed the retardation of growth in *Brachydanio* after thiourea administration to a hypofunctional thyroid. He stated (p. 59), "The evidence obtained from the observation of the glands indicated that such treatment produced a hypofunctional thyroid in the zebra fish." Nigrelli *et al.* (1946), working with *Lebistes reticulatus,* treated simultaneously with mammalian thyroid powder and thiourea, noted an increase in growth of these fish similar to that of their untreated controls and greater than that observed in their thiourea-treated fish. Osborn (1951) studied oxygen consumption by stonerollers (*Campostoma anomalum*) and bigmouth shiners (*Notropis dorsalis dorsalis*) immersed in thiouracil. Oxygen consumption was reduced about 20% with dosages of thiouracil that caused no mortality. The decrease was slight on the first day of treatment, but reached the 20% figure on the second day. A pronounced increase in oxygen consumption took place soon after replacing the fish in plain water. The increase was accomplished more slowly in fish treated for 22 days than in those treated for 9 to 11 days. The BMR is lowered in higher animals treated with goitrogenic compounds (Barker, 1949; Mackenzies, 1943; Leathem, 1945; Gordon *et al.*, 1946). Generally the decrease parallels that seen in surgically thyroidectomized animals. In view of the controversies concerning the fish thyroid, further study of the metabolic maintaining mechanism of these animals should be made.

The ovary of *Lebistes reticulatus* normally contains, after the presentation of young, a number of nearly mature ova (0.8–1.5 mm.) approximately equal to the number of young in previous broods. The number of the smaller and less developed ova (0.3–0.5 mm) is consistently larger than this. This finding indicates that a number of eggs undergo atresia. The thiourea-treated animals of groups 1D, 1W and 2W suffered a decrease in number of young in their fourth brood as well as a smaller than normal number of ova of the sizes mentioned above. Group 3W individuals, although presenting a fourth brood of normal number, possessed ovaries containing fewer than normal eggs of the 0.3–0.5 mm. group. These findings are evidence of a decreased ovarian function. The difference in the severity of reaction to thiourea between the groups is no doubt a reflection of the difference in time of exposure. Chambers (1951), Barrington and Matty (1952) and Scott (1953) also noted a gonad inhibition in fish after thiourea administration. It may be theorized that the decreased ovarian function is the result of a lowered production of a gonad-stimulating hormone elaborated by the pituitary, rather than being a direct action of the administered thiourea. Matthews (1939) has shown that gonadal function in the teleost *Fundulus* is dependent upon the presence of the pituitary. He recorded in 1939 that hypophysectomy results in lowered gonadal activity in that genus. It may well be that with the suppression of thyroid gland activity caused by thiourea, there is a great need for a rise in the titer of a pituitary thyroid-stimulating factor. The increased thyroid-stimulating factor production is accomplished by a sacrificing of the gonad-stimulating hormone to the level where the latter cannot maintain a normal functioning gonad. The present results are comparable to those reported to occur in rats. Barker (1949) noted a markedly reduced fertility with altered estrous cycles after thiourea treatment. Decreases in size and weights of mice ovaries and uteri have been observed by Dalton *et al.* (1945). Jones, Delfs and Foote (1946) induced fetal resorption by thiouracil administration. They also stated that a decrease in the titer of gonadotropin occurred after prolonged treatment with the drug. The small size of the guppy makes hormone analysis of the blood a difficult, if not impossible, procedure at the present time.

It is well known that the secretory activity of the thyroid is regulated by an interaction and balance between that gland and the anterior lobe of the pituitary. A decrease in thyroid hormone output reflects itself in an increased production of the thyroid-stimulating hormone by the pituitary (Gordon *et al.*, 1945a; Grasso, 1946). A hypofunctioning thyroid may be produced by the administration of thiourea and related compounds. It has been found that the ability of the thyroid to take up iodine is depressed within one hour after the injection of anti-thyroid compounds into rats (Larson *et al.*, 1945). As a consequence of the decreased amount of circulating thyroid hormone and an increase in the stimulating factor, the thyroid is changed morphologically and physiologically. The thyroid tissue is a very labile one and soon demonstrates morphological alterations in an attempt to adjust its hormone output. Hyperemia and heightened epithelium are seen within 24 to 48 hours. Hyperplasia becomes pronounced in most forms within a week.

The scheme outlined in the preceding paragraphs, *viz.*, a decreased thyroid function followed by an increased circulating titer of thyroid-stimulating hormone and a decreased amount of a gonad-stimulating hormone, may well explain the interference with reproductive activities seen after thiourea immersion.

The thyroid tissue of all young of all treated groups gave evidence of great stimulation. Heightened cells, afollicular cell arrangement, masses of thyroid tissue without colloid, and metastasis to abnormal sites were found. Two main hypotheses may be offered to explain the stimulation. It may be argued that thiourea itself passed from the blood vessels of the maternal ovarian follicle wall to the embryo by way of the structures previously described. This compound, circulating within the embryo, suppressed the activity of its thyroid, resulting in an increased embryonic thyroid-stimulating hormone titer. The high level of the stimulating factor then caused morphological changes in the target organ. This hypothesis assumes fetal thyroid and pituitary function. The second possible explanation depends upon an increase in adult thyroid-stimulating factor (which must be granted in order to explain the adult thyroid changes) and the transfollicular transmission of this agent from the adult to the embryo. The maternally derived factor then stimulated the embryo thyroid. Valuable information on this question might be obtained if gravid females could be hypophysectomized in the latter part of the gestation period and subjected to thiourea starting at that time. If thiourea and an embryonic pituitary factor were responsible for the hyperplastic thyroid of the young noted in the present work, these same changes would follow this procedure. On the other hand, if the adult pituitary factor was the sole agent causing the hyperplasia the thyroid would not be changed. This method of attack assumes that the females could maintain and deliver their young after being deprived of the pituitary. Considering molecular size alone, other factors being equal, it would be easier for thiourea to pass from the adult to the embryo. The molecular weight of thiourea is 76.12. The thyroid-stimulating hormone in a rather purified form obtained from sheep and beef pituitary glands possesses a molecular weight of approximately 10,000. Because of species differences, this finding cannot be carried over directly to the fish pituitary factor, but it does allow some speculation as to the size of the molecule. Direct placental transmission of anti-thyroid compounds has been shown to occur in the rat (Hughes, 1944; Williams, 1944; Goldsmith *et al.*, 1945) and in the mouse (Kaufman *et al.*, 1948).

Accepting the fact that the *Lebistes reticulatus* embryo is contained within an ovarian follicle and has no connection with the external environment except by way of the adult, one must concede that a transfollicular movement of material is possible and did occur in the present work. The use of the term ovoviviparous, in its strict sense, to describe the guppy is therefore open to question.

Summary

1. The adult thyroid of *Lebistes reticulatus* after exposure, by immersion, to a 0.04% thiourea solution displayed an extreme hyperplasia. An increase in follicle size and number and their establishment in abnormal areas took place. The heart, kidney, spleen, and intestines were invaded by the thyroid epithelial cells. The mean height of the follicular cells of the fish receiving the shortest thiourea treatment increased approximately 130%. With longer periods of exposure to thiourea, greater heights were produced.

2. The thyroid gland of the newborn of thiourea-treated females had undergone hyperplasia and metastasis during ontogeny. The thyroid became afollicular, lost colloid and possessed tall epithelial cells. Hearts, kidneys, spleens and intestines harbored the invading cells. A transfollicular transmission of an agent causing these changes took place.

3. *Lebistes reticulatus,* while not having an anatomical uterus, placenta or other means of attaching the developing embryo firmly to a part of the adult female reproductive system, does have maternal and embryonic modifications which permit an exchange of material. The species is therefore not a true ovoviviparous form.

4. The gametogenic function of the adult ovary was suppressed by thiourea administration as evidenced by abortions, decrease in brood size, longer brood intervals and smaller than normal number of ova in the gonad. This finding may be a reflection of a decreased gonad-stimulating hormone titer. The latter may be due to a shift to a greater thyroid-stimulating hormone production at the expense of a gonad-stimulating hormone by the pituitary.

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